Chapter 4

The Molecular Basis of the Pineal Melatonin Rhythm: Regulation of Serotonin *N*-Acetylation

David C. Klein, Ruben Baler, Patrick H. Roseboom, J.L. Weller, Marianne Bernard, Jonathan A. Gastel, Martin Zatz, P. Michael Iuvone, Valerie Bégay, Jack Falcón, Greg Cahill, Vincent M. Cassone, and Steven L. Coon

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The day/night rhythm in circulating levels of melatonin (*N*-acetyl 5-methoxytryptamine) is a constant characteristic of vertebrate physiology (Figure 4.1);[1] circulating melatonin is always elevated about tenfold at night relative to day values. Melatonin is considered to be the hormone of the night[2] because it provides the organism with a highly reliable humoral signal that is proportional to the duration of the night phase. Changes in night length, such as those which occur on a seasonal basis, are translated into changes in the duration and/or timing of the period of elevated melatonin production. The highly reliable and accurate translation of night length into melatonin production by vertebrates reflects a set of regulatory mechanisms which limit high levels of melatonin production to the night period and minimize synthesis in the light at any time.

Seasonal lengthening and shortening of the nocturnal melatonin signal can globally change physiology in some species, altering reproduction, body weight, behavior associated with reproduction, and coat color.[1,3] In some cases, such as sheep, animals become reproductively active in response to shorter periods of melatonin production; in others, such as the Syrian hamster, this inhibits reproduction. Melatonin has a role in all vertebrates to modulate endogenous clock function; it also influences a broad range of physiological functions, including sleep.[1,3,4]

In sharp contrast to the conserved day/night pattern of melatonin production, there is remarkable species-to-species diversity in both the anatomical organization of the systems which generate this rhythm and in the molecular and cellular mechanisms which control melatonin biosynthesis. Such diversity indicates many solutions have evolved to ensure that the nocturnal increase in melatonin is a reliable indicator of the night period. This emphasizes the essential role the rhythm in melatonin plays in vertebrate physiology.

This chapter describes conserved and species-specific features of melatonin rhythm-generating systems. It is divided into three sections. The first is an overview of the basic functional components of these systems. The second is devoted to a molecule which has unique importance in vertebrate circadian biology — serotonin *N*-acetyltransferase (arylalkylamine *N*-acetyltransferase, AANAT). This enzyme is the critical interface between regulatory mechanisms and melatonin synthesis. This pivotal role has earned it the designation "the melatonin rhythm-generating enzyme". The last section emphasizes the diverse nature of melatonin rhythm-generating systems by describing examples from three vertebrate classes.

4.1 General Characteristics of Melatonin Rhythm-Generating Systems

The fundamental components of melatonin rhythm-generating systems are a site of melatonin production, a source of ~24 hour signals, and a detector through which light acts on the system (Figure 4.2).

4.1.1 The Site of Melatonin Production

The source of circulating melatonin is the pineal gland, which can be considered to be the melatonin factory. The ability of this tissue to produce high levels of melatonin reflects several biochemical features, including high levels of tryptophan hydroxylase (the first enzyme in the conversion of circulating tryptophan to serotonin), a high concentration of serotonin, and high levels of the enzymes required for the serotonin–*N*-acetylserotonin–melatonin pathway, i.e., AANAT and

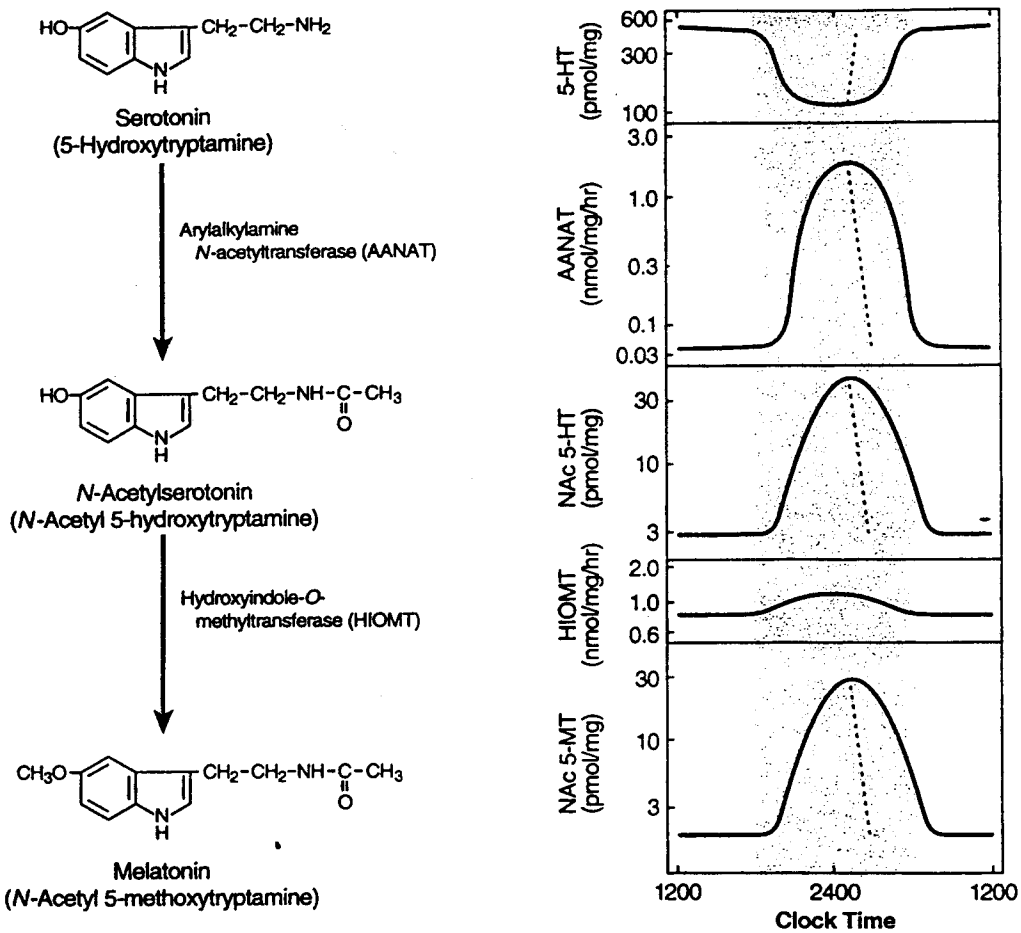

FIGURE 4.1
Rhythms in pineal indole metabolism. The dashed lines at night (shaded) represent the effects of an unexpected exposure to light at night. (From Klein, D.C. et al., *Rec. Prog. Hormone Res.*, 52, 307, 1997. With permission.)

hydroxyindole-*O*-methyltransferase (HIOMT; see Figure 4.1). Other tissues do not possess all these features, and for this reason pinealectomy causes melatonin to nearly disappear from the circulation.[1] As discussed below, the low levels of melatonin synthesized in the retina do not contribute to circulating melatonin.

As indicated above, AANAT is an interface which converts regulatory input into changes in the production of melatonin. Day/night changes in melatonin production occur in response to changes in AANAT activity, which controls *N*-acetylserotonin levels. The rate of melatonin production by HIOMT is a mass action function of *N*-acetylserotonin (Figure 4.1).

4.1.2 *Sources of ~24-Hr Signals*

The rhythmic ~24-hr signals which determine the pattern of melatonin production are generated by one or more clocks. In most lower vertebrates, an internal clock is typically located in the pineal gland. In mammals, the internal clock is in the suprachiasmatic nucleus (SCN) of the hypothalamus. In some vertebrates, such as the chicken, multiple internal clocks control melatonin synthesis.

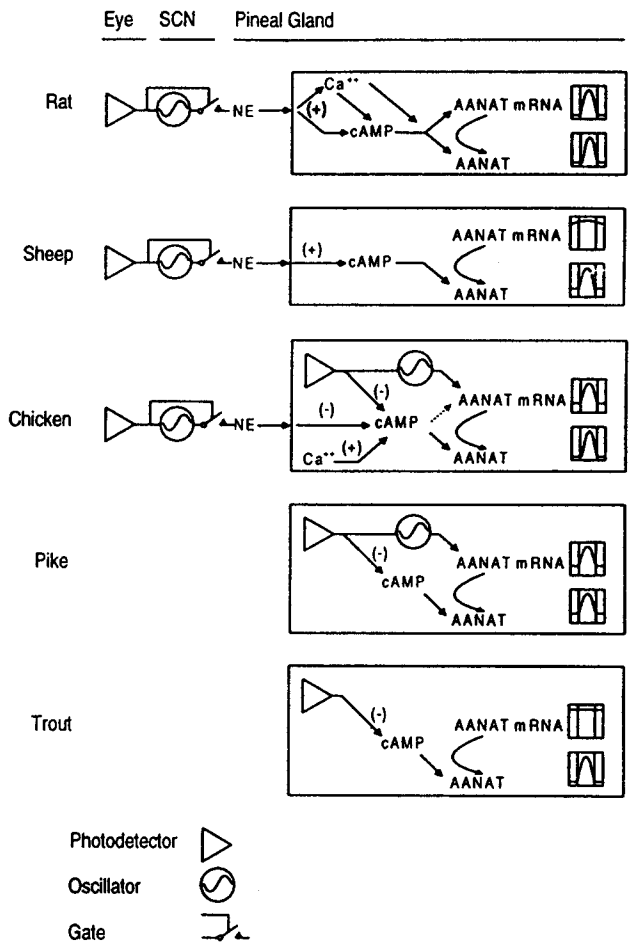

FIGURE 4.2
Melatonin rhythm-generating systems. For details see the text.

Systems that lack an internal clock have also been identified, such as the trout pineal gland. In these cases, the environmental dark/light cycle generates a diurnal rhythm in melatonin production.

One functional advantage of an endogenous clock which drives melatonin synthesis — as opposed to a simple light-off/dark-on system — is that the clock prevents high levels of melatonin synthesis from occurring during the day if animals are in darkness. Another advantage is that it allows an animal to anticipate day/night transitions. In this way, eating schedules and the sleep/wake cycle are optimally synchronized with the environmental light/dark cycle.

4.1.3 Sites of Photodetection and the Effects of Light

The third component of melatonin rhythm-generating systems is the photodetector. Light entrains and modulates the clock and also switches off melatonin production by turning off AANAT activity. The photoreceptors which act on the SCN clock are located in the lateral eyes; photoreceptors which act on pineal clocks are located in the pineal gland.

The entrainment function of light is the main mechanism which resets the clock and synchronizes it with the environmental light/dark cycle. Without this resetting influence, the internal clock

free-runs, i.e., drifts out of phase with the light/dark cycle. The modulation function adjusts the period that the clock stimulates the pineal gland at night, which increases in the winter (long nights) and decreases in summer (short nights). The entrainment and modulation actions of light involve alterations in clock function. Light also acts to suppress melatonin production by interrupting clock stimulation of AANAT. This minimizes synthesis of melatonin during the day and reinforces limitations imposed by the internal clock.

4.2 AANAT — The Melatonin Rhythm-Generating Enzyme

The importance of AANAT in vertebrate circadian biology has stimulated significant interest in the regulation of enzyme activity, the links between pineal clocks and AANAT, the mechanism of enzyme action, and the basis of tissue-specific expression. Our current knowledge of this enzyme is summarized here; a more detailed description is available elsewhere.[5]

AANAT is referred to both as serotonin *N*-acetyltransferase and as arylalkylamine-*N*-acetyltransferase. The former nomenclature reflects the fact that serotonin is the best known substrate of the enzyme; the latter nomenclature recognizes the general chemical family to which serotonin belongs. A small group of other arylalkylamines are also substrates, including tryptamine, methoxytryptamine, phenylethylamine, and tyramine.[5]

Although it is clear that AANAT is the key regulator of the large day/night change in melatonin production, and that day/night changes in HIOMT protein do not appear to play a dominant role in generating this rhythm, it is important to note that melatonin production is subject to limitations imposed by the activity of tryptophan hydroxylase and the availability of serotonin and cofactors, in addition to HIOMT activity.

Two features of the pattern of the serotonin-melatonin pathway are seen in all vertebrates. One feature is the reciprocal relationship between serotonin and the *N*-acetylated derivatives, with high levels of serotonin occurring during the day and low levels at night. The second is the switch-off effect of light, which converts the nighttime pineal indole pattern to a daytime pattern (see the broken line in Figure 4.1). Although this is very rapid in the rat, the rate varies somewhat from species to species.

4.2.1 Tissue Distribution

AANAT is selectively expressed in the pineal gland and to a lesser and more variable degree in the retina.[5,8–12] Melatonin synthesis in the retina is relatively low and is thought to serve a local function.[13–16] Very low levels of AANAT expression have also been detected in brain regions, pituitary, and testes;[5] melatonin synthesis in these sites, however, has not been documented.

It is of interest to note that the retinal expression of AANAT is a reflection of a more general pineal/retinal overlap in gene expression. In some species, both tissues synthesize melatonin, detect light, and contain endogenous clocks. Pineal cells and retinal photoreceptor cells develop from adjacent areas of the roof of the diencephalon, and it is thought that both types of cells share a common ancestral photoneuroendocrine cell capable of phototransduction and circadian synthesis of melatonin.[17,18]

The pineal/retinal pattern of gene expression may be determined in part by genomic photoreceptor conserved elements (PCEs), which are short DNA sequences that identify genes for expression in these tissues.[19] Such elements occur in photoreceptor-related genes in both vertebrates and invertebrates, including opsins, and in the HIOMT and AANAT genes.[20,21] It is suspected that developmental expression of these genes is regulated by PCE binding proteins.

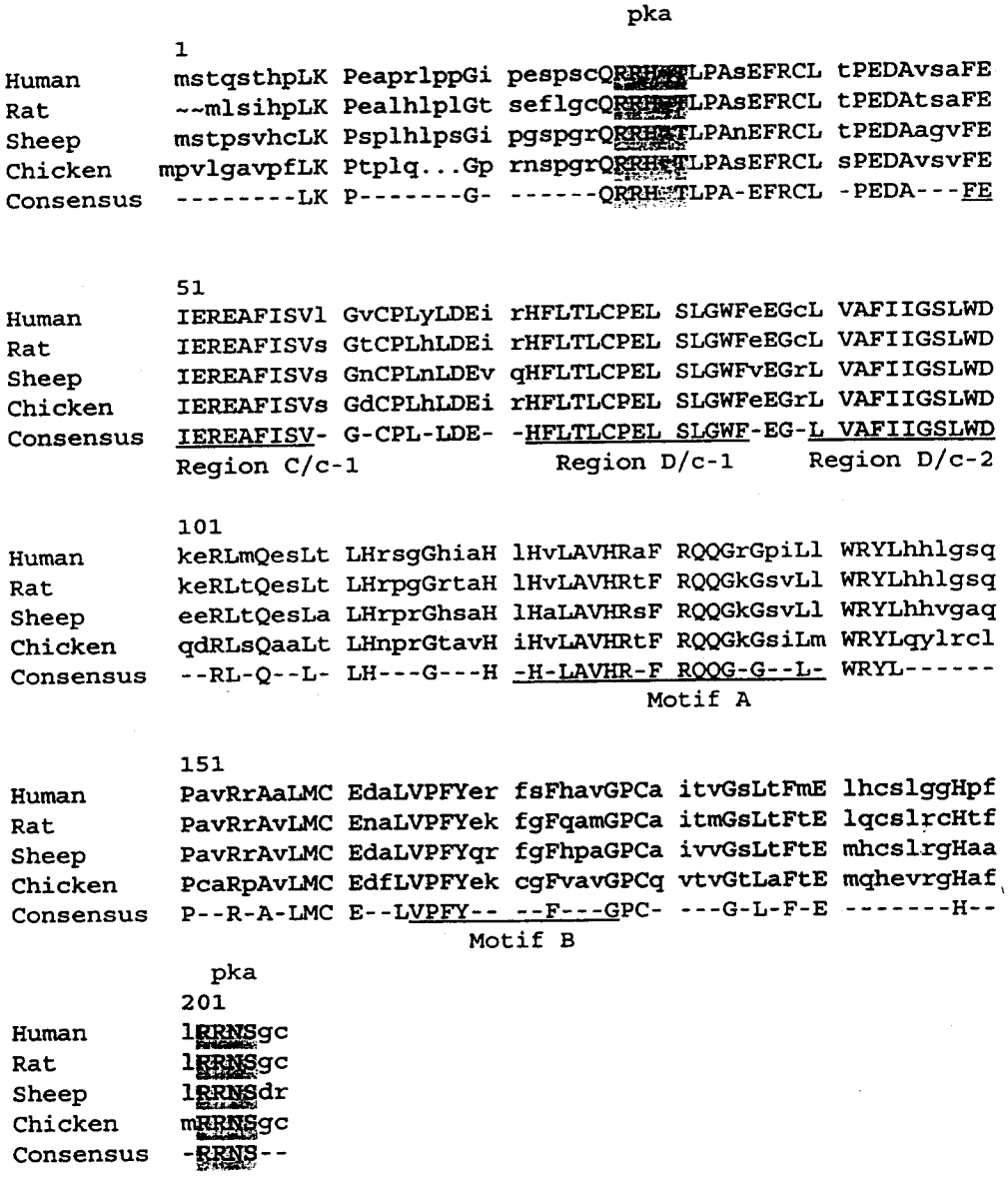

FIGURE 4.3

AANAT amino acid sequences. Deduced amino acid sequences of AANAT from human (GenBank accession # U40347), rat (GenBank accession # U38306), sheep (GenBank accession # U29663), and chicken (GenBank accession # U46502). The consensus sequence identifies amino acids that are identical between all species listed. Capital letters conform to the consensus sequences. The conserved putative cyclic nucleotide-dependent protein kinase phosphorylation sites are shaded. The conserved motifs shared with other members of the A/B (GNAT)[5,8,12a] superfamily are underlined, as are the regions unique to AANATs; the nomenclature assigned to these regions reflects limited homology with C and D motifs identified within the superfamily and conserved (c) AANAT sequences.

4.2.2 Functional Anatomy of AANAT

At this writing, structure/function relationships of the AANAT molecule are under active investigation, and some of the concepts presented here should be regarded as "best guesses". AANAT is a cytosolic ~24-kDa protein (203 to 207 amino acids; see Figure 4.3).[5,8–12] The catalytic domain

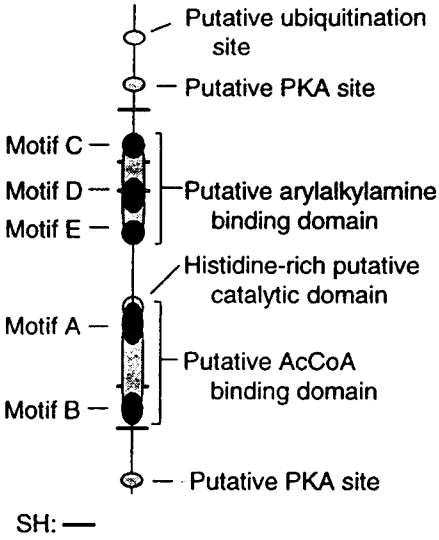

FIGURE 4.4
The functional anatomy of AANAT. The identified features are conserved in all the available deduced amino acid sequences (see Figure 4.3). PKA, cyclic AMP-dependent protein kinase; SH, cysteine. (Modified from Klein, D.C. et al., *Rec. Prog. Hormone Res.*, 52, 307, 1997. With permission.)

(~140 amino acids), in which substrate binding and acetyl transfer occur, occupies the central core of the molecule (Figure 4.4). The apparent AcCoA binding site is characterized by two motifs — motifs A and B, which occur within a 60-residue stretch (Figure 4.3). These motifs are sequences of functionally similar residues, rather than identical amino acids. Their presence in tandem is the identifying feature of the members of a superfamily of acetyltransferases which otherwise exhibit little similarity; the terms A/B and GNAT have been used to identify this superfamily.[5,8,12a] Although all members use AcCoA as an acetyl donor, each member exhibits high specificity toward a distinct narrow set of substrates, such as histones, antibiotics, diamines, or biogenic amines.

The putative arylalkylamine binding domain of AANAT (~50 amino acids) is characterized by three conserved regions (C/c-1, D/c-1, and D/c-2; Figures 4.3 and 4.4). Interestingly, another A/B superfamily arylalkylamine *N*-acetyltransferase has been identified in *Drosophila melanogaster* (DMNAT).[22] It has the very low homology to AANAT as is seen with other A/B superfamily members and does not contain these conserved regions. Accordingly, it does not belong to the AANAT gene family.

The putative catalytic domain which transfers acetyl groups from AcCoA to arylalkylamine acceptors is located between the arylalkylamine and AcCoA binding domains. Acetyl transfer is presumed to reflect the catalytic action of the imidazole moiety of histidines in this region.[5,23]

Two prominent putative regulatory features of the enzyme are the cyclic AMP-dependent protein kinase (PKA) phosphorylation sites in the C- and N-terminal regions of the protein. These sites are suspected of being important because they are conserved in all AANAT molecules and also because cyclic AMP is known to be critical for maintaining AANAT activity in all systems.

The initial 25-amino-acid portion of AANAT bounded by the N-terminal PKA phosphorylation site has a relatively high abundance of prolines and a 100% conserved lysine; otherwise, this region has relatively poor sequence conservation. The conserved lysine could play a very important regulatory role because it may be a site for ubiquitination. This is thought to target proteins for degradation by the proteasome, the macromolecular complex which contains multiple proteolytic activities.[24] The high abundance of prolines in this region may also play a role in this process. The presence of the PKA site and the lysine in this region suggests that they mediate adrenergic-cyclic AMP-regulated proteolysis, as discussed below.[24]

4.2.3 Regulation of AANAT Activity by Synthesis and Proteolysis

A very close relationship exists between AANAT protein and activity.[24] This is evident at all times and persists during the light-induced turn-off. This is of importance in understanding the regulation of AANAT because it eliminates the possibility that large changes in activity reflect posttranslational modifications that shift existing populations of AANAT molecules between active to inactive forms. The requirement for *de novo* synthesis for activity to increase makes the light-induced turn-off a one-way "off-only" switch.[24,25] This prevents spikes in melatonin synthesis at inappropriate times in response to transient, sporadic fluctuations in second messengers. Such spikes might occur if activity were only regulated by a simple posttranslational event, such as phosphorylation. This mechanism enhances the integrity of the melatonin rhythm-generating system by reducing "noise".

Cyclic AMP plays at least two roles in regulating AANAT, each of which differ in relative importance on a species-to-species basis. It appears likely that in all species cyclic AMP regulates activity by blocking AANAT proteolysis. This might function as the sole regulator of enzyme activity in species in which AANAT mRNA levels are constantly elevated. A hypothetical scenario is that AANAT protein is always made when AANAT mRNA is available and that cyclic AMP acts to inhibit proteolysis of newly synthesized molecules of AANAT. Cyclic AMP might influence degradation through a direct influence on AANAT phosphorylation or through an indirect influence via phosphorylation of a protein involved in targeting AANAT for degradation. For example, cyclic AMP might inhibit the hypothetical conjugation of AANAT to ubiquitin, or it might activate deubiquitination of ubiquitinated AANAT. In pineal glands in which AANAT mRNA is always available, cyclic AMP inhibition of proteolysis allows AANAT protein and activity to increase immediately at the start of the night — without a lag — and to be maintained at a high level throughout the night. As a result, melatonin can be produced from dusk to dawn in most natural lighting cycles.

The second mechanism through which cyclic AMP controls AANAT activity is by regulating AANAT mRNA. As described below, AANAT mRNA is nearly undetectable during the day in the rat, and cyclic AMP induces a 100- to 300-fold increase. Without this increase, AANAT activity cannot increase. The advantage of this is that it eliminates the possibility that AANAT activity and melatonin production could increase during the day. It is not unreasonable to suspect that in cases where there is a rhythm in AANAT mRNA, inappropriate production of melatonin does not favor species survival.

It should be added that in addition to cyclic AMP, other factors play a role in regulating AANAT activity. These include calcium and unidentified factors which link the pineal clock to expression of the AANAT gene.[1,2,5,10]

4.3 Examples of Melatonin Rhythm-Generating Systems

The following section covers pineal melatonin rhythm-generating systems but will not cover how retinal melatonin synthesis is controlled.[27,28] Although similarities in melatonin production exist between the pineal gland and retina, this is not always the case. For example, in the hamster, mouse, and rat, the retina differs from the pineal in that the former contains a clock, whereas the latter does not.[29] In this way, the rodent retina appears to be similar to the retinae and pineal glands of lower vertebrates.

4.3.1 Regulation in Mammals

4.3.1.1 Organization of the Mammalian Melatonin Rhythm-Generating System

The anatomical organization of the rhythm-generating system is essentially identical in all mammals. The clock that drives pineal melatonin synthesis is in the SCN,[30] which is connected to the

pineal gland by a neural pathway passing through central and peripheral structures.[25] SCN cells project to cells in the paraventricular nucleus, which in turn send projections down the spinal cord to the intermediolateral cell column and synapse with preganglionic cells. These innervate superior cervical ganglia cells which send norepinephrine (NE)-containing sympathetic projections to the pineal gland. At night, stimulatory signals from the SCN cause the release of NE into the pineal extracellular space. Photic signals act via the retina and travel to the SCN via a retinal hypothalamic projection which exits the optic nerves at the optic chiasm.[25,31]

Light at night acts downstream of the SCN clock to block release of NE in the pineal gland. This effect is enhanced at the level of the pineal gland by the rapid uptake of residual extracellular NE into sympathetic nerve terminals; light is not known to act directly on the mammalian pineal gland.

4.3.1.2 Adrenergic Signal Transduction

The most important positive influence of NE is β_1-adrenergic stimulation of adenylate cyclase.[25] This is potentiated by simultaneous stimulation of α_1-adrenergic receptors which elevates intracellular Ca^{++} ($[Ca^{++}]_i$) and increases the activity of several phospholipases and activates protein kinase C.[25,32] Activation of protein kinase C is primarily responsible for sensitizing adenylate cyclase[33] to β_1-adrenergic activation. The resulting increase in cyclic AMP is essential for the increase in AANAT activity; $[Ca^{++}]_i$ also appears to act in an independent manner to enhance downstream effects of cyclic AMP.[33]

4.3.1.3 Features of AANAT Regulation in the Rat

Cyclic AMP acts to regulate AANAT protein and activity in the rat by increasing AANAT mRNA accumulation and by preventing proteolysis.[9,24,25] The time course of the nocturnal increase in melatonin production in the rat, as is true also of the hamster,[1,34] is characterized by a lag phase followed by a sigmoidal shaped response which returns to basal values prior to lights-on.

4.3.1.3.1 ***Transcriptional Regulation.*** Cyclic AMP increases AANAT mRNA 100- to 300-fold, from nearly undetectable levels through PKA phosphorylation of a cyclic AMP response element binding protein (CREB). CREB resides on the AANAT gene, bound to a cyclic AMP response element (CRE).[9,35,36] The consequent increase in AANAT mRNA is accompanied by an increase in AANAT protein and activity, provided adrenergic stimulation is maintained. In contrast to AANAT protein and activity, AANAT mRNA does not decrease rapidly when stimulation is abruptly blocked by light exposure or adrenergic blockade.

The amplitude of the increase in AANAT mRNA appears to be governed in part by the inducible cyclic AMP early repressor (ICER), a negatively acting transcription factor which competes with CREB for binding to the CRE.[37] Although the mRNA encoding this protein exhibits a dramatic rhythm in abundance similar to that of AANAT, ICER protein is relatively stable and does not undergo dramatic day/night changes. It is reasonable to suspect that ICER protein provides an integrated molecular memory of the duration of previous night periods and that this influences future patterns of response, by limiting the transcriptional response.[38]

Rat pineal AANAT mRNA gradually decreases at the end of the night. This may be due to several redundant mechanisms. First, it is generally thought that the strength of SCN stimulation starts to decrease late in the night. Second, it is likely that the cyclic AMP response of pineal cells to adrenergic stimulation gradually decreases during the course of the night, due to down-regulation and desensitization. A third possible negative influence is the induction of the early immediate gene, Fos-related antigen-2 (Fra-2). Each night Fra-2 protein increases rapidly, with dynamics similar to those of AANAT mRNA and AANAT protein.[39] Fra-2 heterodimerizes with a member of the Jun family to form a complex which is thought to bind strongly to AP-1 sites in the AANAT promoter, yet not induce transcription. As a result, it may suppress transcription.

The presence of significant levels of AANAT mRNA only at night, as is the case in the rat, essentially restricts AANAT protein synthesis to the night. The time required for AANAT mRNA to accumulate imposes a lag on the timing of the increase in AANAT protein and activity. This may be of special functional importance in fine-tuning the shape of the melatonin signal in seasonal breeders with short gestation periods, such as the hamster,[34] where very subtle changes in the duration of the night period control reproduction.[1]

4.3.1.3.2 ***Regulation by Inhibition of Proteolysis.*** The second important regulatory mechanism through which cyclic AMP acts in the rat is to prevent proteolysis of AANAT protein. As described above, when cyclic AMP is high, AANAT appears to be long-lived and not subject to significant proteolysis. However, when cyclic AMP drops, both activity and protein drop in parallel.[24] The half-life of the decrease in activity and protein in the rat is approximately 3.5 min. It is also likely that cyclic AMP-inhibition of protein degradation permits AANAT protein to accumulate when AANAT mRNA increases and also maintains elevated levels of AANAT protein at night.[24]

4.3.1.3.3 ***Unidentified Influences of Cyclic AMP.*** The current model of how cyclic AMP regulates AANAT protein and activity in the rat is based on the dynamics of mRNA and the regulation of proteolysis. It proposes that AANAT protein and activity increase when mRNA is available and proteolysis in inhibited. However, other mechanisms may play a role. For example, cyclic AMP might hypothetically enhance AANAT translation.[21]

4.3.1.4 Features of Regulation in Sheep

Sheep are representative of those species in which the melatonin rhythm has a square wave pattern, i.e., melatonin increases rapidly after lights off. Other mammals exhibiting this pattern include the human and monkey.[1,40] AANAT activity is regulated by an adrenergic-cyclic AMP mechanism in sheep[41] as it is in the rat. However, regulation in sheep differs from that in rats in that AANAT mRNA is always high — day and night (Figure 4.2). It is reasonable to speculate that the primary mechanism regulating melatonin synthesis is the cyclic AMP inhibition of AANAT proteolysis. Day levels of AANAT activity in sheep are higher than those in the rat, probably due to higher daytime values of AANAT mRNA in the daytime sheep pineal gland.

4.3.2 Regulation in Chicken

The best-studied model of regulation of pineal AANAT activity in birds is the chicken.[42] There are a number of interesting differences between regulation of melatonin production in the chicken and in mammals (Figure 4.2).

4.3.2.1 Organization of the Melatonin Rhythm-Generating System in the Chicken

Two clocks drive the melatonin rhythm in birds. One is located in the SCN and the other in the pineal gland.[43] The SCN clock appears to provide negative influences on melatonin production, mediated by the release of NE during the day. The increase in melatonin appears to reflect the combined effect of an increase in cyclic AMP and of independent influences of the pineal clock on AANAT mRNA.[10,26] Light acts on the system through two routes, the retinal-SCN system and through pineal photoreceptors.

4.3.2.2 Cellular Regulatory Mechanisms

The negative influence of the SCN clock on pineal function in the bird appears to be mediated by NE, acting through α_2-adrenergic receptors to decrease cyclic AMP levels during the day. This maintains low levels of AANAT activity and melatonin production during the day. It is interesting to note that although light suppresses AANAT activity, it does not suppress the clock-driven rhythm in AANAT mRNA.[10]

The positive influences which increase melatonin at night reflect an increase in AANAT mRNA and cyclic AMP. The increase in AANAT mRNA is driven by the pineal clock, without a strong role of cyclic AMP;[26] the link between cyclic AMP effects and clock effects on functional expression of the AANAT gene is not well understood.[2,44] However, a dark-associated increase in cyclic AMP promotes the increase in AANAT activity, and it is reasonable to suspect that this reflects an increase in AANAT protein due to cyclic AMP-dependent inhibition of AANAT proteolysis.

In addition to cyclic AMP, $[Ca^{++}]_i$ plays an important role in regulation of AANAT activity and melatonin production. $[Ca^{++}]_i$ can influence cyclic AMP production and, via this mechanism, alter AANAT activity. Furthermore, there is clear evidence that $[Ca^{++}]_i$ plays a role in phase shifting the pineal clock.[2,44] Photic regulation of chicken pinealocyte $[Ca^{++}]_i$ has not been established, although this does appear to occur in the retina.[27]

4.3.3 Regulation in Fish

As is true of the chicken, the pineal glands of most fish have an endogenous clock which drives melatonin production (Figure 4.2).[46,47] This makes the fish pineal gland an excellent, yet somewhat overlooked, model for the study of circadian mechanisms.

4.3.3.1 Pike and Zebrafish

The translation of the pineal-clock-driven rhythm in AANAT mRNA in the pike and zebrafish pineal glands[12] into changes in AANAT activity and melatonin production are controlled by light acting through cyclic AMP.[46] Although it has not yet been determined that changes in activity are due to changes in AANAT protein nor that inhibition of proteolysis is involved, these seem to be reasonable hypotheses to pursue. Cyclic AMP follows a diurnal bimodal rhythm in pike, with peaks occurring at the L/D and the D/L transitions.[47] These variations are circadian in nature, yet it is not known whether they are controlled by a clock or are part of the clock mechanism, nor is it known in which cells the increase in cyclic AMP is occurring. However, it is reasonable to propose that the increase in AANAT expression and activity is due to the increase in cyclic AMP which occurs at the L/D transition. The functional importance of the D/L associated increase in cyclic AMP is unclear.

4.3.3.2 Trout

Trout are an example of a melatonin rhythm-generating system that lacks an endogenous clock in their pineal gland.[48–51] Rather, the trout pineal gland responds to darkness and light directly without the imposition of a clock. As a result, a dark-on/light-off relationship to melatonin production can be demonstrated at all times, day and night. Other species with this type of regulation include lizards and the lamprey eel.[52,53]

Trout pineal AANAT mRNA levels are continually elevated, and it appears that AANAT activity increases in the dark when cyclic AMP is high and decreases in the light which causes a decrease in cyclic AMP. Ca^{++} also appears to play a role in the control of AANAT because $[Ca^{++}]_i$ parallels melatonin secretion. $[Ca^{++}]_i$ increases in the dark as a consequence of photoreceptor depolarization, which triggers the opening of voltage-gated Ca^{++} channels.[54] Conversely, light

exposure hyperpolarizes the cells, the channels close, and $[Ca^{++}]_i$ decreases. The effects of $[Ca^{++}]_i$ are mediated by cyclic AMP-dependent and -independent mechanisms.[55]

4.4 Final Comments

This chapter has reviewed the molecular basis of melatonin rhythm-generating systems in vertebrates. The conserved and the species-specific features of these rhythms described here represent a rich body of information. The reader is encouraged to obtain a more thorough and detailed description of the unique features of these systems from the original reports and reviews cited here and to ponder the interesting question of the functional advantages of the unique features of these systems. In some cases, the species-specific differences, such as the role of transcriptional regulation, could serve primarily as a mechanism which tailors the melatonin production signal and regulates the lag period between the onset of night and the rise of serum melatonin. In other cases, the adaptive advantages are not obvious. Future research in this area should enable us to better link the diverse modes of regulation to the role of melatonin in each species; in addition, our understanding of the role of melatonin in vertebrate physiology may be improved. Finally, an understanding of the molecules and molecular mechanisms involved in the regulation of melatonin production will provide new pharmacological targets for drugs which modulate circadian rhythms.

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